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Multidrug resistant *Mycobacterium tuberculosis* amongst Category I & II failures and Category II relapse patients from Pakistan

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ABSTRACT

Objective: To determine the prevalence of multidrug-resistant tuberculosis (MDR-TB) among previously treated TB patients in Khyber Pakhtunkhwa (KP) Province, Pakistan.

Design and settings: A cross-sectional study was conducted (January–September 2009) in 10 districts of KP. All Category (CAT) I and CAT II failures, and CAT II relapse cases were recruited within 1 week following declaration of treatment outcome or re-registration of CAT II. Clinical information and sputum was collected from each patient.

Results: Total 139 patients were enrolled. *Mycobacterium tuberculosis* bacilli (MTB) was isolated in 113 (81.3%) samples; Mycobacterium other than tuberculosis (MOTT) was isolated in 7 (5%) samples. MDR-TB was noted in 66 (58.4%) patients and extensive drug resistant (XDR-TB) in 2 (1.8%) patients. Amongst MDR patients, 20 (62.5%) were CAT I failure, 19 (76%) CAT II failure and 27 (48.2%) CAT II relapse cases. Resistance to Isoniazid was most common in 84 (74%) cases, followed by Pyrazinamide in 73 (64.6%) cases, Rifampicin in 67 (59%) cases, Streptomycin in 60 (53%) cases, Ethambutol in 58 (51%) cases, and Ofloxacin in 18 (22.2%) cases.

Conclusion: High rate of drug resistance, including MDR observed among failures and relapse cases. This study emphasizes the need to review TB care delivery, particularly in failure cases in difficult regions such as KP that have seen considerable population displacement and conflict in recent years.

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Abbreviations: AKU, Aga Khan University; AGEG, AGEG Consultants; ATT, anti-TB drugs treatment; CAT, Category; CLSI, Clinical Laboratory Standard Institute; DST, Drug Susceptibility Testing; DTO, District TB Officers; DOTS, Directly Observed Therapy Short course; E, Ethambutol ; XDR-TB, extensive drug resistant TB; FATA, Federally Administered Tribal Areas; GTZ, German Technical Cooperation; H, Isoniazid; IQR, inter-quartile ranges; KP, Khyber Pakhtunkhwa ; KNCV, Koninklijke Nederlandse Centrale Verneniging Tot Bestrujding Der Tuberculose; LJ, Lowenstein Jensen; MDR-TB, multidrug-resistant TB; MOTT, Mycobacterium other than tuberculosis; MTB, *Mycobacterium tuberculosis* bacilli; OR, Odds Ratio; PTP, Provincial TB Control Program; Z, Pyrazinamide; R, Rifampicin; SPSS, Statistical Package for Social Sciences; S, Streptomycin; SD, Standard deviation; STCP, Strengthening the Tuberculosis Control Programme; TB, tuberculosis; WHO, World Health Organization; PANTA, Polymyxin B, Amphotericin B, Nalidixic acid, Trimethoprim and Azlocilin; AGEG/GTZ STCP, AGEG/GTZ Strengthening the Tuberculosis Control Programme in North West Frontier Province/Federally Administered Tribal Areas Pakistan

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Introduction

Tuberculosis (TB) is a major public health problem, with 9.4 million new cases and 1.7 million deaths each year worldwide [1]. Pakistan with an annual incidence rate of 231/100,000 population and a prevalence rate of 355/100,000 population, ranks 8th in the estimated global TB burden list [1,2]. Pakistan is one of the 27 high multidrug-resistant MTB (MDR MTB) burden countries with estimates of 2.9% MDR in untreated and 35.4% amongst previously treated TB patients [3]. The emergence of MDR-MTB, i.e. resistance to at least Rifampicin and Isoniazid associated with treatment failure, relapse, complications and mortality, presents additional challenges in TB control [4,5]. The response of patients with MDR-MTB to other first-line anti-tuberculosis treatment (including Pyrazinamide and Ethambutol) is poor and results in high case fatality rates. Treatment of these patients with second-line drugs is on the other hand expensive and toxic, often requiring hospitalization for the management of complications arising as a result of the therapy used and its side effects [4,6,7]. Management of MDR cases, therefore, adds additional constraints onto the limited health care resources of developing countries [8,9]. Moreover, emergence of extensive drug resistant TB (XDR-TB) further complicates treatment with reduced efficacy of available anti-tuberculosis drugs [10–12]. Poor compliance with DOTS guidelines and inadequate care delivery resulting in treatment failure and relapse is a major cause of drug resistance in tuberculosis [3,4].

While a limited number of studies on drug resistance in tuberculosis have been carried out in laboratory settings, prevalence of MDR-TB among previously treated TB cases in Pakistan is unreported [10,13,14]. Such information would be useful not only to estimate the extent of the problem in this high-risk population, but also for reviewing the tuberculosis control program and for developing the necessary steps for preventing the spread of MDR-TB in the country. TB care is particularly challenging in the Khyber-Paukhtunkhwa province (KP), which harbors internally displaced persons and has a long history of conflict. A study was therefore conducted to determine the prevalence of MDR TB among previously treated TB cases, i.e., failure cases of CAT I and failure cases and relapses of CAT II patients in KP.

Materials and methods

A cross-sectional study was conducted in KP province of Pakistan from January 2009 to September 2009. KP province borders Afghanistan to the north-west, Gilgit-Baltistan to the north-east, Azad Jammu and Kashmir to the east, the Federally Administered Tribal Areas (FATA) to the West and South, Baluchistan to the south and Punjab and the Islamabad Capital Territory to the south-east. The main ethnic groups in the KP province are the Pashtuns, locally referred to as Pakhtuns, who form about two-thirds of the population, followed by a number of smaller ethnic groups, most notably, the Hindkowans. The KP province has an estimated population of roughly 21 million, excluding the 1.5 million Afghan refugees and their descendants in the province [15]. Pulmonary tuberculosis patients fulfilling the World Health Organization (WHO) diagnostic criteria for CAT I (patients who have never received treatment for TB) and CAT II (retreatment of, relapse, treatment failure, smear positive who have taken anti-tuberculosis treatment (ATT) for more than one month and defaulted) [16] were recruited from 10 selected districts of KP. Selection of the 10 districts was based on the frequency of failure cases of CAT I and CAT II patients during 2006 and during the first half of 2007, availability of a reachable Aga Khan University (AKU) collection point and the prevailing security situation. Failure cases were selected within 1 week by District TB Officers (DTO)/Directly Observed Therapy Short course (DOTS) facilitators of the diagnostic unit. During a period of 9 months, sputum was collected from all CAT I failure cases and CAT II failure and relapse cases within 1 week after the declaration of treatment outcome or re-registration of CAT II patients. Early morning sputum specimens were collected from the patients and transported to the nearest AKU collection point as soon as possible. In case of delay, the sputum samples were stored in a refrigerator and transported within 24 h. Drug resistance testing for anti-tuberculosis drugs was performed according to standard Clinical Laboratory Standard Institute (CLSI) guidelines [17]. Clinical information forms were filled out by trained DOTS facilitators, the District TB Officers (DTO) or the treating physician. Adequate training was given to the staff through the Provincial TB Control Program (PTP) with the involvement of the respective regional TB control program officers. Information was collected for district, sex, age, weight, marital status, education, occupation, number of person living in the family, any person with TB in the family, clinical history and treatment history.

Laboratory methods

Sputum samples were decontaminated using *N*-acetyl-L-cysteine (NALC) sodium hydroxide and then concentrated using centrifugation (3000×*g*) for 30 min. Sediment was used for AFB microscopy and culture. Smears for microscopy were screened using Auramine staining, and positive slides were confirmed by Kinyoun modification of Ziehl Neelsen stain [18]. Lowenstein Jensen (LJ) medium (Oxoid) and MGIT were used for culture. For LJ slant 0·2 ml of concentrated specimen was inoculated and incubated for up to 8 weeks or until positive if earlier. MGIT vials were inoculated with 0·5 ml of specimen and incubated at 37 °C after supplementation of medium with PANTA; containing Polymyxin B, Amphotericin B, Nalidixic acid, Trimethoprim and Azlocilin. Growth index of inoculated vials was checked for up to 8 weeks or until positive earlier. Growth from the positive LJ slant, and MGIT vials were first stained with Kinyoun and *Mycobacterium tuberculosis* identified by BACTEC NAP TB differentiation test (Becton Dickinson, USA). Nitrate reduction and Niacin positivity were also determined [19]. Susceptibility testing was performed using an agar proportion method on enriched Middlebrook 7H10 medium (BBL) at the following concentrations; Rifampicin 1 μg/ml and 5 μg/ml, Isoniazid 0·2 μg/ml and 1 μg/ml, Streptomycin 2 μg/ml and 10 μg/ml, Ethambutol 5 μg/ml and 10 μg/ml, Ethionamide 5 μg/ml, Capreomycin 10 μg/ml,

Amikacin 5 μg/ml, Kanamycin 6 μg/ml, and Ofloxacin 2 μg/ml. Pyrazinamide sensitivity was carried out using the BACTEC 7H12 medium pH6·0 at 100 μg/ml (BACTEC-TM PZA test medium, Becton Dickinson, USA). MTB H37Rv was used as a control with each batch of susceptibility testing. For purposes of this study, the following concentrations were used for analysis; Rifampicin 1 μg/ml, Isoniazid 0·2 μg/ml, Streptomycin 2 μg/ml, and Ethambutol 10 μg/ml.

MDR-TB was defined as *M.tuberculosis* strains with resistance to at least Isoniazid and Rifampicin. XDR-TB was defined as MDR-TB with additional resistance to Ofloxacin and at least one of the following injectable drugs: Kanamycin, Capreomycin, or Amikacin. Drug-resistant tuberculosis is defined as *Mycobacterium tuberculosis* bacilli (MTB) resistant to one or more first line anti tuberculosis drugs. These include Rifampicin (R), Isoniazid (H), Ethambutol (E), Streptomycin (S) and Pyrazinamide (Z). Failure case is defined as a patient who is started on re-treatment regimen after having failed previous treatment. Relapse case is defined as a patient previously treated for TB who has been declared cured or treatment completed, and is diagnosed with bacteriologically positive (smear or culture) tuberculosis [16].

Data management and analysis

Data was entered in the Microsoft Excel (database) and then transported to SPSS version 16 for analysis. Means (±SD) were calculated for normally distributed quantitative variables; median with inter-quartile ranges (IQR) was reported for quantitative variables with unknown distribution. Frequencies with (%) were computed for categorical variables. Prevalence of MDR pulmonary tuberculosis was calculated separately in all failure categories. To evaluate the association between MDR-TB status and all of the categorical patient and environmental factors, chi-square test of independence was applied, and for continuous variables (age and weight) *t*-test applied. If chi-square and *t*-test showed significant associations (p-value < 0.05), then strength and direction of the association was determined through logistic regression technique.

Results

A total of 139 patients were enrolled in the study. MTB was isolated in 113 (81.3%) patients; mycobacterium other than tuberculosis (MOTT) in seven (5%) patients; while 19 (13.7%) were culture negative. All patients with culture proven MTB were included (Table 1). These included 32 (28.3%) CAT I failure patients, 25 (22.1%) CAT II failure patients and 56 (49.6%) CAT II relapse patients. The mean age of study subjects was 31.3 (±14.7) with a male to female ratio of 1.05. The majority (61.5%) of the subjects were married and only 29.5% had received primary education. The unemployment rate was high at 84.5%; and the majority of the patients came from large families, with 49.1% reporting other TB patients in their families.

MDR MTB was noted in 66/113 patients, including 20 CAT I failure, 19 CAT II failure and 27 CAT II relapse cases. Resistance to Isoniazid (H) was seen most frequently in 74% of the patients, followed by resistance to Pyrazinamide (Z) in 64.6%, Rifampicin (R) in 59%, Streptomycin (S) in 53% and Ethambutol (E) in 51% of cases (Table 2). Resistance to Ofloxacin was seen in 22.2% of the TB strains. Resistance to Kanamycin, Amikacin, Capreomycin and Ethionamide was not detected in CAT I failure patients. MDR plus Fluoroquinolone resistance was observed in 18/113 cases. Only two (1.8%) isolates were XDR MTB (Table 2).

No significant difference was observed in the drug resistant patterns of CAT I failure and CAT II failure. Marginal statistical significance was observed for Isoniazid (p-value; 0.067) and Pyrazinamide (p-value; 0.091) between CAT I failure and CAT II relapse cases. On the other hand, significantly higher resistance was noted for Isoniazid (p-value; 0.007), Rifampicin (p-value; 0.029), Pyrazinamide (p-value; 0.024), and MDR (p-value; 0.020) in CAT II failure as opposed to relapse cases (Table 2). None of the demographic variable was found to be significantly associated with MDR status on chi-square and *t*-test, therefore advanced measures i.e., Odds Ratio (OR) calculated through logistic regression technique, were not reported here.

Discussion

An MDR-TB rate of 58% amongst failure and relapse cases is in agreement with other studies carried out in the country also showing high MDR prevalence linked to acquired drug resistance [20–23]. These findings are also consistent with regional studies reporting 47–56% MDR in previously treated patients [22–24].

The high resistance to Isoniazid in all three categories is extremely concerning, and points to an increasingly limited role of this essential drug in such patients. The high level of Isoniazid resistance is however consistent with other studies [20,21,24,25,27–30].It is encouraging, however, that resistance to second line agents was not seen in CAT I patients. The presence of fluoroquinolone resistance, included in 28% of CAT I failures though alarming is consistent with a previous study of AKUH [31]. Other regional studies have also reported similar trends of increasing fluoroquinolone resistance in MDR isolates [32,33].

In the study population, prevalence of drug resistance, including multidrug resistance, was significantly higher among failures as compared with relapse cases. The present study findings of higher resistance among treatment failures is consistent with other regional studies; a study from New Delhi, India, observed 50% MDR in failure cases and a 27% MDR strain in relapse cases [24]. Among retreatment patients, higher resistance has also been reported among treatment failures in Ahmedabad (70%) and Bangalore (78%) compared with 50.7% and 50% in relapsed patients, respectively [25,26]. These results underline the need for timely identification of treatment failures by early referral for culture and sensitivity testing and early initiation of appropriate treatment with second-line drugs.

In this study, although statistical analysis was hampered by the small sample size, MDR patients tended to have lower education levels, higher unemployment rate, and report overcrowding and presence of a TB contact within their families. This serves to highlight the at risk population requiring additional support.

Table 1 – General characteristics of all culture positive TB subjects and comparison of characteristics of MDR and Non MDR pulmonary tuberculosis cases (*n* = 113).

Patients characteristics	Total (*n*%)	MDR TB (*n*%)	Non MDR TB (*n*%)	*p*-value
	113 (100)	66 (58.4)	47 (41.6)	
Age (mean ± SD)	31.265 (14.69)	31.409 (14.702)	31.064 (14.849)	0.903
Weight (mean ± SD)	45.477 (11.24)	45.733 (11.773)	44.754 (10.598)	0.651
Gender				
Female	55 (48.7)	32 (58.2)	23 (41.8)	0.962
Male	58 (51.3)	34 (58.6)	24 (41.4)	
[c]Marital status				
Single	40 (38.5)	22 (55)	18 (45)	0.212
Married	64 (61.5)	43 (67.2)	21 (32.8)	
Education				
No formal schooling	68 (60.2)	43 (63.2)	25 (36.8)	0.400
Primary	34 (30.1)	18 (52.9)	16 (47.1)	
Secondary or higher	11 (9.7)	5 (45.5)	6 (54.5)	
[b]Currently employed				
No	82 (84.5)	48 (58.5)	34 (41.5)	0.708
Yes	15 (15.5)	8 (53.3)	7 (46.7)	
No. of persons in the family(mean ± SD)	10.48 (7.41)	10.65 (7.25)	10.4 (7.32)	0.664
[a]TB patient in family				
No	55 (50.9)	34 (61.8)	21 (38.2)	0.454
Yes	53 (49.1)	29 (54.7)	24 (45.3)	
Failure				
Failure CAT I	32 (28.3)	19 (62.5)	11 (37.5)	0.055
Failure CAT II	25 (22.1)	19 (76)	6 (24)	
Relapse CAT II	56 (49.6)	27 (48.2)	29 (51.8)	

[a] *n* = 5 Missing.
[b] *n* = 16 Missing.
[c] *n* = 12 Missing.

Table 2 – Resistance pattern in different failure and relapse categories.

Drug Susceptibility Testing (DST)	Total *N* (%)	Failure CAT I *N* (%)	Failure CAT II *N* (%)	Relapse CAT II *N* (%)	*p*-value
	113 (100)	32 (28.3)	25 (22.1)	56 (49.6)	
Susceptible to all five first line drugs	21 (18.6)	5 (16.6)	1 (4)	15 (26.8)	0.017
Any resistance					
Isoniazid	84 (74.3)	26 (81.2)	23 (92)	35 (62.5)	0.007
Rifampicin	67 (59.3)	20 (62.5)	19 (76)	28 (50)	0.029
Pyrazinamide	73 (64.6)	23 (71.9)	20 (80)	30 (53.6)	0.024
Ethambutol	58 (51.3)	18 (56.2)	14 (56)	26 (46.4)	0.426
Streptomycin	60 (53.1)	19 (59.4)	13 (52)	28 (50)	0.868
[a]Ofloxacin	18 (22.2)	7 (28)	3 (13.6)	8 (23.5)	0.363
[a]Kanamycin	2 (2.5)	0 (0)	1 (4.5)	1 (2.9)	0.752
[a]Amikacin	3 (3.7)	0 (0)	2 (9.1)	1 (2.9)	0.318
[b]Ethionamide	1 (1.5)	0 (0)	0 (0)	1 (3.7)	0.422
[a]Capreomycin	2 (2.5)	0 (0)	1 (4.5)	1 (3)	0.769
MDR	66 (58.4)	20 (62.5)	19 (76)	27 (48.2)	0.020
MDR + Fluoroquinolone	18 (15.9)	7 (21.9)	3 (12)	8 (14.3)	0.279
XDR	2 (1.8)	0 (0)	1 (4)	1 (7.1)	0.798

p-values are given for comparison of failure CAT II and Relapse CAT II cases.
[a] *n* = 23 For Failure CAT-I, *n* = 22 for Failure CAT-II, *n* = 34 for relapse CAT-II (except Capreomycin *n* = 22) and 81 for total (except Capreomycin *n* = 80).
[b] *n* = 21 For failure CAT-I, *n* = 17 for failure CAT-II, *n* = 27 for relapse CAT-II and 67 for total.

In Pakistan to date, tuberculosis is mostly diagnosed on clinical suspicion and on therapeutic response to anti-tuberculosis drugs, rather than on the basis of culture isolation [29]. Such practices result in the inappropriate use of anti-tuberculosis drugs and poor compliance with treatment. Despite TB having been declared a national emergency in 2001,

implementation of the National TB Control Program has been hampered by under-developed health facilities and limited resources. Regions such as Khyber Pakhtunkhwa face the added challenge of interrupted patient access due to conflicts and/or natural disasters.

Conclusion

This study, including all CAT I and CAT II failures, as well as CAT II relapses registered in the study area and therefore representative of the population, emphasizes the need to review TB care delivery, particularly in failure cases in at risk population particularly in difficult regions such as KP. This study also emphasizes the importance of continuous monitoring of drug resistance trends, which would be helpful in shaping future policies to prevent the emergence and dissemination of MDR.

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Ethical consideration

Verbal consent was taken from all the study subjects.

Author's contribution

Abdul Ghafoor (AG), Nek Dad Afridi (NA), Hans-Uwe Wendl-Richter (HW) and Rumina Hasan (RH) planned and conducted the study. Jaishri Mehraj (JM) analyzed data and drafted the manuscript under the supervision of Rumina Hasan (RH). Yasraba Rafiq (YR) coordinated the study, contributed to data management and analysis, and in manuscript writing. All authors have read and approved the final manuscript.

Conflict of interest

The authors declare that there are no conflicts of interest.

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